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(11)

EP 1 162 278 A3

(12)

EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
14.04.2004 Bulletin 2004/16

(51) Int Cl.7: C12Q 1/68

(43) Date of publication A2:
12.12.2001 Bulletin 2001/50

(21) Application number: 01304958.0

(22) Date of filing: 06.06.2001

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR
Designated Extension States:
AL LT LV MK RO SI

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(30) Priority: 08.06.2000 US 209987 P
23.05.2001 US 862417

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(54) Isometric primer extension method and kit for detection and quantification of specific nucleic acid

(57) A method for detecting and/or quantifying a target DNA or RNA present in a sample by a isometric primer extension method is disclosed. The method includes carrying out a primer extension reaction in the absence of a free nucleotide so that the primer extension reaction is stopped where the absent nucleotide would have been inserted. Thus, as the amount of incorporation of a labeled nucleotide on the primer extended product is detected, the amount of the target RNA or DNA in the sample is measured.

Fig. 1

Target DNA Detection and Quantification

(1) Extension with dTTP, dGTP, dCTP, either with ddATP or without dATP

(SEQ ID NO.2)
5' TGATCAGAGCTGAATCTGCTGATTCGACGACGCGGAGATTCTGCTCTTAAAGCGGATAGCAT 3'
3' attctcag Primer 2 (SEQ ID NO.3) Primer 1

(2) Extension with dATP, dGTP, dCTP, either with ddTTP or without dTTP

5' TGATCAGAGCTGAATCTGCTGATTCGACGACGCGGAGATTCTGCTCTTAAAGCGGATAGCAT 3'
3' attctcag Primer 4 (SEQ ID NO.4) Primer 3

(3) Extension with dATP, dGTP, dTTP, either with ddCTP or without dCTP

5' TGATCAGAGCTGAATCTGCTGATTCGACGACGCGGAGATTCTGCTCTTAAAGCGGATAGCAT 3'
3' ttctag Primer 5 Primer 6

Line represents primer sequence. Small letters a, g, c, and t are bases extended from the primer, some of which are labeled with a detectable marker. u and t are used interchangeably.



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EUROPEAN SEARCH REPORT

Application Number
EP 01 30 4958

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The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 4 February 2004	Examiner Knudsen, H
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 03/02 (P4/C01)



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The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 4 February 2004	Examiner Knudsen, H
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03.02 (P/4C01)



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Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT			
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The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (In.CI.7)
Place of search MUNICH		Date of completion of the search 4 February 2004	Examiner Knudsen, H
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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



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**LACK OF UNITY OF INVENTION
SHEET B**

Application Number

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The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-6,11-40 (partly), 7-9 (completely)

This invention concerns a primer extension method using one or two or three types of non-terminator nucleotide with or without a terminator nucleotide that is different from the non-terminator nucleotides wherein either the non-terminator nucleotide or the terminator nucleotide is labelled and the amount of labelling signal is detected or quantified on the primer extended nucleotides.

2. Claims: 1-6,11-40 (partly), 10 (completely)

This invention concerns a primer extension method using one or two or three types of non-terminator nucleotide with or without a terminator nucleotide that is different from the non-terminator nucleotides wherein the amount of extended primers is detected by mass spectrometry.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

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